VOLUME XLII NUMBER 4

BOTANICAL GAZETTE

OCTOBER, 1906

THE DEVELOPMENT OF AGARICUS CAMPESTRIS.*

GEO. F. ATKINSON.

(WITH PLATES VII-XII)

IN some respects the history of the study of the Hymeniales does not present the same progress which can be seen in the other groups of fungi, or indeed in nearly all other groups of plants. The earliest period, that of the study and classification of species and genera, presents in the main the same aspects which have been characteristic of the early study of all plants; but the progress made up to the present time is not in proportion to the time and energy expended, due to certain difficulties, some inherent in the nature of the plants themselves, and others due to the lack of an adequate knowledge of their anatomy and development.

The second period, that of the study of the morphology and development, began more than half a century ago. It is true that in the early part of the 19th century, nearly a century ago, quite an elaborate theory of the development of the Hymeniales, especially the Agaricaceae, was evolved by NEES VON ESENBECK.[1] But his theory, embellished as it is with his philosophical ideas of the evolution and metamorphosis of these plants from the puffballs and truffles; in which he was evidently influenced by the philosophy expressed in the *Vorwort* of GOETHE'S *Farbenlehre*, that in a book dealing with

* Contribution from the Department of Botany of Cornell University. No. 110. Paper presented before Section G, of the A. A. A. S., at the New Orleans meeting.

[1] Das System der Pilze und Schwämme, ein Versuch von Dr. C. O. NEES VON ESENBECK, I-XXXVI, 1-329, 44 plates, Würzburg, 1816. See also NEES VON ESENBECK, Plantarum Mycetoidarum in Hort. Bonn. obs. evolutio. Nov. Act. Natur. Curios. 16: pars 1. 1832, for development of *Agaricus volvaceus*.

natural phenomena the writer should make use of a lively imagination in order to make it real to the reader; and especially because there is such lack of definiteness as to the forms studied, though it is quite evident he refers more especially to species of Amanita, presents little that is helpful to the present discussion.

At that early period it was an important forward step to show, as DUTROCHET[2] did in 1834, that the large fungi were only the fruit bodies of the plants then known as "Byssus," which spread usually underground or in the substance of organic bodies; and for TROG[3] in 1837 to recognize the two different parts in the life history, the vegetative stage or mycelium and the fruiting stage or carpophore, and that this is the product of germinating spores; though MICHELI had stated as early as 1729[4] that the fruit bodies of some fungi did not come immediately from the seed (spores), but the seeds first produce a large root which grows for several years in the ground, and then gives rise to the fruit body (referring to *Polyporus tuberaster*). But during the early and middle portion of the 19th century the work on the morphology and anatomy of these plants, and the descriptions and illustrations of species, was far in advance of the work on development and the organization of the parts of the fruit body. Unfortunately the study of the morphology and development of the Hymeniales has not kept pace with the same studies in other groups of plants. J. SCHMITZ[5] studied the very early stages of five different species. While the work appears to be very carefully done for that early time, it does not meet modern requirements; and while his results perhaps in the main are not far out of the way for the species studied (*Cantharellus sinuosus*, *C. tubaeformis*, *Cortinarius bulliardi* (Pers.) Fr., *Coprinus niveus*, *Hydnum imbricatum*), it will be seen later he was led into a mistake in formulating a general law, based on these five species, to apply to the development of all the pileate fungi.

[2] Mém. **2**:173. 1834.

[3] Ueber das Wachsthum der Schwämme, Flora **20**:609. 1837.

[4] Nova plantarum genera 134. 1729.

[5] Mycologische Beobachtungen, als Beiträge zur Lebens- und Entwickelungsgeschichte einiger Schwämme aus der Klasse der Gastromyceten und Hymenomyceten. Linnaea **16**:141–215. *pls. 6–7.* 1842. See especially the part II, Ueber die Bildung neuer Theile bei den Hymenomyceten, vorzugsweise den Pileaten. Idem 168–179.

BONORDEN[6] deals briefly with the anatomical structure of the genera recognized by FRIES in his *Epicrisis*. He does not discuss the differentiation of the parts in the young fruit body, but describes somewhat in detail the different forms of the universal veil, its mode of dehiscence, and its relation to the partial veil and the pileus in certain species of Lepiota (pp. 178–181). He says, very briefly (p. 8): "From the mycelium rises the fruit body of the fungus (stroma, thallus), either naked from the first or enclosed in an envelop (velum, volva). The latter consists always of very much elongated cells, tubes, which are like the tissue of the mycelium, and has therefore always a structure very different from that of the fruit body of the fungus; it is to be regarded as a continuation of the mycelium. The envelop is ruptured by the further growth of the pileus and is thrown off, but sometimes remains a part of the same and forms the epidermis of the pileus, on which account this is so different in structure in the case of the gastromycetes and pileated fungi from the other parts of the fungus."

H. HOFFMAN contributed at that time some important work on the anatomy and morphology, and as early as 1856 gave a very brief account of the origin of the hymenium of *Agaricus campestris*.[7] In speaking of his studies of the developmental history of the lamellae in very different types (*l. c.*, p. 145) he cites three extremes: *Agaricus carneo-tomentosus* (*Panus torulosus*), where they arise at the apex of the young fruit body; *Agaricus campestris*, where they originate deeper in the interior and develop laterally; and *Hymenogaster klotzschii*, where they remain concealed in the interior of the fungus. In describing the development of *Agaricus campestris* (p. 145) he says (I give a free translation): "It begins, as BULLIARD has already very well represented (Champ. d. France. *pl. 514, fig. L.* 1791–1809), in the form of small spheres which for a part rest upon thick mycelium strands. This stroma is formed as in the former case [*Agaricus carneo-tomentosus*, the stroma of which, he says, at first quite homogeneous, is formed out of felted filamentous cells]. Gradually the same takes on an elongate form; the interior cells grow in a perpen-

[6] Handbuch der Allgemeine Mycologie 147–196. 1851.

[7] Pollinarien und Spermatien bei Agaricus. Bot. Zeit. 14:137–148, 153–163. *pl.* 5. 1856.

dicular direction, the upper ones grow laterally, and then bend abruptly downward; the ends of these cells abjoint a parenchyma which forms the beginning of the lamellae; the under surface of these young lamellae is somewhat even and has no longer any connection with the stroma tissue lying close beneath, which later sinks down as a ring. The hymenial layer opens here round about at the side."

A few years later[8] he describes the development of a number of additional forms, all of which were gymnocarp except one, *Marasmius oreades*, and he remarks (p. 401) that this peculiarity of the internal origin of the hymenium is characteristic of many other Agaricaceae, as was shown in his larger work[9] which appeared in the following year.

The work of HOFFMANN was followed by DEBARY'S[10] study of the development of several species of Agaricaceae and Gastromycetes, by HARTIG'S study of *Agaricus melleus*,[11] by BREFELD'S studies on species of Coprinus,[12] and by the incomplete study of a large number of Agaricaceae by FAYOD[13] nearly twenty years ago, which was the last serious attempt (which I have as yet seen) at a study of the development of the Agaricaceae. Even these few studies do not agree in the account of development which they give of the same or related species. Furthermore, they are very incomplete and unsatisfactory, owing either to the methods employed (freehand sections by the earlier students), or in the scanty material at hand, which did not provide sufficient numbers of the early stages of development or a sufficiently full series of stages.

The difficulties in method are now overcome, but the other difficulties, those of obtaining sufficient material in all stages of development, are still serious in the great number of species. This is because

8 HERMANN HOFFMANN, Beiträge zur Entwickelungsgeschichte und Anatomie der Agaricineen. Bot. Zeit. **18**:389–395, 397–404. *pls. 13–14.* 1860.

9 Icones Analyticae Fungorum, Abbildungen und Beschreibungen von Pilzer mit besonderer Rücksicht auf Anatomie und Entwickelungsgeschichte 1–105. *pls. 1–24.* 1861.

10 Morphologie und Physiologie der Pilze, Flechten und Myxomyceten 68. 1866.

11 Wichtige Krankheiten der Waldbäume, etc. 25. 1874.

12 Unters. a. d. Gesammtgebiet d. Mycol. **3**:13–122. *pls. 1–9.* 1877.

13 Prodrome d'une histoire naturelle des Agaricinées. Ann. Sci. Nat. Bot. VII. **9**:181–411. *pls. 6–7.* 1889.

of the fact that at present few species have been cultivated artificially (except in the genus Coprinus by BREFELD, *l. c.*) so as to obtain the stages of development, and because the feral species nearly all pass their early and critical stages of development within the substratum and therefore are difficult to find, and at the same time it is often difficult to be certain to what species they appertain. These difficulties have probably played an important part in discouraging the further study of development of the Agaricaceae.

It is rather surprising, however, that even in the present time we do not have a sufficiently clear, full, and accurate account of the development of the fruit body of *Agaricus campestris*, especially the origin and differentiation of the various parts of the plant. This is the more so because this species is so common and of such wide distribution, but especially because it has been cultivated for so many years under conditions in which large numbers of carpophores in all conceivable stages of development are so easily obtained. Perhaps the very commonness and richness of the material has been the chief reason of its having been passed by.

Having given some attention to the study of the Agaricaceae for several years, especially as to their economic and biologic significance, as well as to the recognition of species and genera, the need of studies of development has been brought very forcibly to my attention, and I have been obtaining material for this study in several different genera. The meager and conflicting accounts which we have of the development of *Agaricus campestris*, as well as the ease with which material can be obtained, has led me to deal with this species first.

DEBARY[14] says the fruit body of many Agaricaceae (*Agaricus campestris*, *A. praecox*, *Coprinus micaceus* and relatives) is in its early youth a body interwoven out of delicate, dense, and uniform hyphae. At a very early stage, through differentiation of the original homogeneous weft, the principal parts of the fruit body are outlined and limited. On the interior of the upper part of the body a small and narrow air space of the form of a horizontal ring arises through the separation of the tissue elements. The portion which lies above becomes the pileus, the tissue present surrounded by it and below it

[14] Morphologie und Physiologie der Pilze, Flechten und Myxomyceten 68. 1866. Leipzig.

becomes the stem. The tissue on the outside of it corresponds to the edge of the pileus, but its hyphae continue without interruption or change into the outer surface of the stem below.

In 1874 R. HARTIG,[15] in his study of the development of *Agaricus melleus*, says that "the investigation of the earliest condition of the fruit body of *Agaricus melleus* shows that here, as well as in the Agaricaceae not provided with a veil, the pileus arises through a superficial annular furrow which in the beginning is completely open to the outside, and that later through growth of the marginal hyphae of the pileus and of the stem the annular furrow becomes covered over with a hyphal layer, the veil." He further says that if one compares his *fig. 20* (which shows the veil covering the hymenial tract) with DEBARY'S *fig. 26* (*l. c.*) of the young *Agaricus campestris*, "it appears from the agreement of the two figures that the conjecture is justified that also by this last fruit body in the region of the hymenial tract a subsequent growing together of the hyphae of the pileus and stem has taken place." Longitudinal sections of the young fruit body of *Agaricus campestris* at this stage do give the suggestion that the veil originates as HARTIG described for *Agaricus melleus*. DEBARY evidently did not study the very young stages of *Agaricus campestris*, for all his figures of longitudinal sections show the veil covering the hymenial area. It seems that without reinvestigating the question he adopted HARTIG'S suggestion that the development in *Agaricus campestris* followed the same course as that described by HARTIG for *Agaricus melleus*. In his later work[16] he says in reference to those forms with a "marginal veil" (*velum partiale* of FRIES): "Up to the first formation of the pileus on the summit of the stipe-primordium the phenomena are the same in essential points as in the gymnocarpous forms" (referring to his figures of *Collybia dryophila*, p. 55). "The young pileus is entirely delimited from the stipe by a transverse annular furrow running along its future hymenial surface. But then the superficial hyphal layers of the stipe and of the young pileus send out numerous branches toward one another from the edges of the furrow; these unite into

15 Wichtige Krankheiten der Waldbäume, etc. 25. *pl. 106.* 1874.

16 Comparative morphology and biology of the fungi, etc. 289–290. 1887. English edition.

a close weft, the marginal veil, which bridges over the furrow and closes it on the outside" (referring to figure of *Agaricus melleus* copied from HARTIG). To be certain that DEBARY here refers also to *Agaricus campestris* I quote also from page 291: "Most marginal veils are formed in the same way as that of *Agaricus melleus*, and *fig. 132* of *A. campestris* will serve to illustrate these remarks." *Fig. 132* is reproduced from his original work in 1866. In addition, on page 295 he says: "The account given above of the development of the species which are furnished with a marginal veil is founded, wherever it departs from my former statements, chiefly upon the facts discovered by HARTIG and BREFELD;" and on page 297, after discussing the different types of development in the Agaricaceae and his former statements, he says: "So far as these statements related to Coprinus they have been shown by BREFELD's researches to be incorrect; my own did not pay sufficient regard to the earliest stages of development. I will not even maintain that they are quite correct for *Agaricus campestris*, ... but readily allow that the facts in the case are always the same as in *A. melleus*, and that the first extension of the marginal veil over the hymenial surface which was originally exposed had there also been overlooked."

GOEBEL[17] says: "These veil-formations are connected with the entire growth of the fructification; the species with a naked pileus are by their nature gymnocarpous." In speaking of the young fructifications of *Agaricus campestris*, he says: "These are at first pear-shaped bodies composed of young uniform hyphae, and each of these bodies is a rudimentary stipe, from the upper part of which the pileus will be developed. At an early period the hyphal tissue gives way in such a manner as to form an annular air cavity beneath the summit of the stalk, this cavity enlarges with the growth of the whole body, its upper wall forming the under side of the pileus, from which the radial hymenial lamellae grow in downward direction and fill up the cavity." His account thus supports DEBARY's earlier account, but the evidence presented in illustrations is not sufficiently convincing in view of the controverted nature of the question and especially in view of the fact that gross anatomy and freehand sections

[17] Outlines of classification and special morphology of plants 132–134. 1887. English edition.

do not show the early stages of organization and differentiation, and GOEBEL'S figures (*fig. 89, l. c.*) of the section of the young fruit bodies are made after the young pileus is differentiated from the stipe. No evidence is given that this text book account is the result of original investigation, and it is more likely that GOEBEL here is relying on the early account given by DEBARY, and the still earlier one given by HOFFMANN cited above.

In a similar way, in describing the early stages of development of *Agaricus campestris*,[18] I have followed DEBARY'S later account as follows (p. 7): "At the same time a veil is formed over the gills by threads which grow from the stem upward to the side of the button, and from the side of the button down toward the stem to meet them. This covers up the gills at an early period." Aside from the extensive work of FAYOD in 1889,[19] little work seems to have been done on the development of the young sporocarps of the Agaricaceae since the publication of DEBARY'S work in 1887.

In January 1905 an excellent opportunity presented itself for obtaining material in the required stages of development. Cultures had been made in boxes in the greenhouse of the commercial variety of *Agaricus campestris* known as Columbia, sold by the Pure Culture Spawn Company of Missouri. In many cases large numbers of young carpophores were formed at the surface of the substratum which were clean and in excellent condition for study. Preliminary examinations were made by freehand sections, and by staining in order to determine the age and size of the fruit bodies which should be prepared. In fruit bodies about 1^{mm} in diameter there was no evidence of a superficial annular furrow nor of any internal differentiation. Fruit bodies, therefore, from 1^{mm} to 4^{mm} were selected, fixed in chromo-acetic acid, imbedded in paraffin, microtomed, and stained, some in acid fuchsin and some in methyl blue.

The youngest stage is the primordium of the carpophore, a homogeneous body composed of slender uniform dense hyphae, intricately interwoven, and surrounded by a very thin layer of hyphae of a

18 Studies in American fungi, mushrooms, edible, poisonous, etc., 1st edition, Ithaca, N. Y., 1900; 2d ed. 1901; and New York 1903.

19 Prodrome d'une histoire naturelle des Agaricinées. Ann. Sci. Nat. Bot. VII. **9**:181–411. *pls. 6–7.* 1889.

looser and less dense arrangement. This layer is the "universal veil." It is quite distinct in the young stages of these cultivated varieties, continues to increase in extent until the parts of the fruit body are differentiated and the young pileus and stem are manifest by external differences in form. Then it ceases to grow and is torn apart into white floccose patches on the pileus, as will be seen later. In the very young primordia then there is no evidence of a differentiation into stem and pileus in any of the many individuals which I have examined. As the primordia become slightly larger and older, but still before there is any evidence on the outside of an annular furrow or of any differentiation into pileus and stipe, longitudinal sections which are stained show two small deeply stained internal areas near the upper end of the young fruit body and some distance in from the surface. The hyphae here are not yet differentiated, but are richer in protoplasm, showing the origin of a new and special center of growth. This area is an annular one within the fruit body. Very soon afterward this area increases somewhat in extent and many hyphae begin to grow from the upper portion of this area downward. This is the primordial layer of the hymenium. It first arises when the tissue of the fruit body is homogeneous and compact except for the loose thin envelop. The hyphae which grow downward at this early stage are quite characteristic. They are slender and terete, tapering out into a long slender point. This enables them to pierce between the other hyphae of the compact fruit body. In fact at this time there are similar hyphae in the more central and upper portions, where the stem and pileus are to be differentiated, but in no other place at this time is there a definite center of growth which indicates the organization of any special part. These hyphae, partly at least, provide for intercalary growth of the young fruit body. Soon after the hyphae in the primordial layer of the hymenium begin to grow downward, there is a cessation of growth just below this area which results in the rupture and separation of the hyphae at this point in a corresponding internal annular area, forming the well known "gill cavity," which at first is very minute.

This annular primordium of the hymenium marks the differentiation of the primordium of the fruit body into the primordium of the pileus and that of the stem and veil, the latter being the tissue of

the young fruit body external to the hymenial primordium and continuous with what is to be the margin of the pileus above and with the undifferentiated stem surface below. The downward growing hyphae now take on a different form. They are still slender, but are even and blunt and are very densely crowded side by side, are very rich in protoplasm compared with the hyphae of the rest of the young carpophore and consequently take a deep stain.

This first growth takes place on the under side of the young pileus primordium. The vegetative activity in this region of the young pileus is very great, and is very soon extended outward on the periphery or margin of the young pileus, as shown by the very rapid radial growth of the hyphae at the margin of the pileus, but still some distance in from the surface. This radial growth is also accompanied by a very strong hyponastic growth, so that the threads curve downward, and soon it is so strong that the margin of the pileus is strongly incurved, the hyponastic growth appearing to be stronger at the margin and near it than further inward.

At the same time the primordium of the marginal veil increases by intercalary growth. In the participation of the hyphae at the margin of the pileus in the formation of the veil, they seem to show a greater activity in growth so far as the density of the growth and richness in protoplasm is concerned; while the larger portion toward the stem, also increasing by intercalary growth, becomes looser by the rapid elongation of its elements and their partial separation, thus forming numerous small air spaces. This seems to have an important bearing on the supply of fresh air to the young forming hymenium where the air spaces become continually larger, and the first air space formed underneath the annular primordium of the hymenium not only gives place for the development of the latter but also provides aeration. Thus while the veil serves the purpose of protection to the young hymenium, its structure is such as to provide aeration also. After the differentiation of the hymenial primordium, the lateral growth of the pileus is accentuated so that it becomes broader than the stem portion, and now is formed the external annular furrow. Very soon after the hymenial primordium is organized, the tissue of the pileus primordium, or end of the fruit body, takes on a deeper stain in an area extending inward and some depth below

the surface, showing that the pileus primordium is becoming definitely organized through the central portion (*fig. 4*), also sometimes seen in freehand sections in older stages as a compact area (*fig. 15*).

In the very early stage of the hymenial primordium the under surface is even, but very soon the outlines of the gills begin to form by the more rapid downward growth in radial lines. Very soon after the young gills begin to form, the surface of the stem is differentiated. This occurs in such a way as to show that the surface of the stem portion of the young fruit body does not become the surface of the stem except at its extreme lower portion, which probably corresponds to the bulb even when a bulb is not manifest as a thicker portion of the stem. From a point at the junction of the original annular primordium of the hymenium, or the junction of the young stem with the pileus at the inner end of the gills, and then extending obliquely downward and outward, the hyphae take on more active intercalary growth and a richer content of protoplasm. This marks the origin of the cortex of the stem, and distinguishes it very sharply from the elements of the veil and from the internal tissue of the stem which is to be the medulla by a deeper color in stained preparations; also in fresh freehand sections it is usually very clearly seen with the unaided eye as a whitish more compact area which shows well in ordinary photographs at this stage.

This study shows very clearly that the hymenium in *Agaricus campestris* is endogeneous in its origin, as HOFFMANN described so early as 1856 (*l. c.*), and that DEBARY's first account of the development of this species in 1866 was correct so far as it went; but he did not succeed in obtaining for study carpophores sufficiently young to enable him to speak with certainty after HARTIG had thrown doubt on his conclusions as a result of his study of *Agaricus melleus*.

Not only is the hymenium endogenous in its origin, but the differentiation of the stipe and pileus is simultaneous; that is, the initial stages of the stipe and pileus as distinct structures are organized and made evident in longitudinal sections by the origin of the hymenial primordium. In all the sections that I have examined of this species at this stage, there is no evidence of differentiation of the pileus from the stem before the earliest evidence of the hymenial primordium. We should not conclude, however, this mode of development

is necessarily to be found in other plants until they have been studied, though it is probable that it is true for at least some of the other species of Agaricus (Psalliota). FAYOD found that usually the primordium of the pileus was organized in the apex of the homogeneous young carpophore before the appearance of the primordium of the hymenium, and this seems to be true in certain species of Hypholoma studied by one of my students. In fact FAYOD asserts that this can be accepted as a general law. The primordium of the pileus is the first to appear in the organization of the parts of the carpophore from its primordium. It is shown by the area of hyphae composing it taking on a deeper stain in sections. It is in the form of an inverted bowl convex above, concave below, and *Agaricus rubellus* (*Psalliota rubella*) is one of the forms which he studied. The only exceptions which he admits are the coriaceous forms like Lentinus (*l. c.*, p. 296). In this respect these cultivated forms of *Agaricus campestris* show also an exception to this rule, and the primordium of the pileus is to be regarded as diffuse in the primordium of the carpophore, as he suggests for the coriaceous forms.

The question of the simultaneous organization of the pileus and stipe from a young homogeneous fruit body is an interesting one in view of the different theories held by some of the earlier students. Thus FRIES[20] said: "Omnia organa simul, nulla subevolutione nova successive explicantur. Omnes extremitates ipsius Fungi explicati jam in aetate juvenili adsunt," *i. e.*, "all organs are unfolded simultaneously, none by new successive development. All parts (or extremities) of the unfolded fungus itself are already present in the young stage." He thus believed that in the very young fruit body the organs or parts, though rudimentary and invisible, were all present, their manifestation and expression was a matter of unfolding.

This interesting conception is shown also in another place (*l. c.* 40), where he expresses his view of the general mode of growth of the fungi as compared with that of the algae as follows: "Fungi in omnes plerumque directiones a centro, quod junius, sese expandunt," *i. e.*, "Fungi, though young, expand from the center in almost all directions." All the parts being present, the growth in the center pushed them outward, as they enlarged, into their respective positions, while

[20] E. FRIES, Systema orbis vegetabilis, part 1, 40, 43.

the growth in the case of the algae was at the periphery—"explicantur ita, ut extremitates semper sint juniores" (*l. c.* 53).

Then as to the relative time, or priority of the shaping of the different parts, FRIES says (*l. c.* 44): "Pileus v. c. in Agarico formatus est prius quam stipes pronascitur. Stipes enim pilei, cum quo contiguus, prolongatio et receptaculi pars," *i. e.*, "the pileus, for example, in Agaricus is formed before the stipe is produced. The stipe certainly is a prolongation of the pileus with which it is contiguous, and part of the receptacle."

J. SCHMITZ[21] in his *Mycologische Beobachtungen*, as a result of the study of several forms, holds an entirely different view. He agrees that according to his own observations in many cases all parts of the fungus are formed and present in a very young stage. But he cannot understand, nor believe, that these parts are formed at once, that is, simultaneously with the origin of the fruit body as if by the touch of a fairy wand ("wie durch einen Zauberschlag," p. 174). That at certain young stages all parts are to be considered present and yet invisible he regards as belonging to the domain of pure speculation, a hypothesis suited to a philosophical mode of representation or idealization, and not corresponding to reality. SCHMITZ gives an account of his studies on *Cantharellus sinuosus*, *C. tubaeformis*, *Agaricus bulliardi* Pers. (=*Cortinarius bulliardi* [Pers.] Fr.), *Coprinus niveus*, and *Hydnum repandum*, and believes he is justified in formulating for the pileate fungi a law of development as follows: "1st, that a successive formation of single parts or organs takes place; 2d, that this appearance of new parts rises upward just as gradually as in the case of the higher plants, in such a way that the higher standing parts naturally come to view later than the lower parts, that the matrix or hypothallus appears before the stipe, the stipe before the pileus, and the pileus before the hymenium.[22] In *Agaricus* (*Crepidotus*) *variabilis*

[21] Ueber die Bildung neuer Theile bei den Hymenomyceten, vorzugsweise den Pileaten. Linnaea **16**:168–179. 1842.

[22] "Ich glaube also, dass man bei der Pileaten als Gesetz aufstellen darf, dass 1) eine successive Bildung der einzelen Theile oder Organe vor sich gehe; 2) dass dieses Hervortreten der neuen Theile ebenso graduell aufwärts steige, wie bei den höhern Pflanzen, so nämlich, dass die höherstehenden wesentlichen später als die untern zum Vorschein kommen, also die Matrix vor dem Stipes, dieser vor dem Pileus und der letztere (an und für sich) vor dem Hymenium auftrete."

OERSTED[23] says that the stem is produced first, and afterward the pileus.

In the forms of *Agaricus campestris* studied here, as I have shown, the young homogeneous fruit body (*figs. 1, 2*) shows no differentiation into parts (except the rudimentary universal veil), and it is to be considered as the primordium of the carpophore. It is not a stem, nor is it a pileus, there is no differentiation to show even the rudiments or primordia of stem or pileus; there is no more active growth manifest in one place than in another, and no separate group of hyphae with richer protoplasmic content which gives a differential stain. It cannot therefore be considered as a rudimentary stem, as GOEBEL (*l. c.* 132–138) has suggested. It is true we might speak of a stem end and a pileus end, but the application of these terms to the portions of the carpophore primordium which are later to be organized into pileus and stem primordia does not predicate their existence before organization takes place. But soon the differentiation takes place by the appearance of the primordium of the hymenium, which at once delimits also the primordia of pileus, stipe, and marginal veil (*figs. 3–5*). This condition answers well to the conception of FRIES that all parts are present in the young stage, though he conceived them to be present in the still younger stages, which we find is not the case.

DECANDOLLE[24] says that in the case of Agaricus the upper part or pileus develops before the lower part, or stipe. Without critical study of the very young stages one might be led to this conclusion by an examination of certain large pileate short-stemmed forms, and perhaps DECANDOLLE examined such plants. According to FAYOD (*l. c.* 279–280) the pileus is differentiated first, the hymenium

[23] OERSTED, A. S.: Iagttagelser anstillede i Löbet af Vinteren 1863–64, som have ledet til Opdagelsen af de hidtil ukjendte Befrugtningsorganer hos Blapsvampene.—Oversigt over det Kongelige danske Videnskabernes Selskabs Forhandlinger, p. 11. *pls. 1–2.* 1865. Copenhagen.

See translation, Observations made in the course of the winter of 1863–64, which have led to the discovery of the hitherto unknown organs of fructification in the Agaricini by A. S. OERSTED. Quart. Jour. Microscop. Sci. **8**: 18–26. 1868.

[24] "Dans plusieurs, tels que les Agarics, la partie supérieure, qu'on nomme le chapeau, paraît développée avant l'inférieure, qu'on a comparée à une tige ou à un pédoncle; l'inverse semble avoir lieu dans les Clavaires qui paraissent croître de bas en haut." Organographie végétale **1**: 384. 1827.

and stipe later, and it is interesting to note that W. G. SMITH[25] in his study of *Coprinus radiatus* says that the cells of the pileus and the hairs which form the veil are the first to appear (*l. c.* 62), but his study does not seem to have been exact, and a comparison of such a form as Coprinus with Agaricus (Psalliota) is not pertinent at present except as it bears on the attempt of some to formulate a general law of growth.

If we now turn to the law formulated by SCHMITZ for the order of succession of the different parts of the pileate fungi, we see that *Agaricus campestris* does not conform to it, but that it is more in accordance with the idea expressed in the first sentence quoted from FRIES, that all parts of the fungus unfold simultaneously. This must not, however, be taken wholly to support FRIES' conception of the young sporocarp nor his idea of central growth. While it is likely that a number of other fungi will be found to agree with *Agaricus campestris* in the mode of organization of the parts of the plant from the primordium of the sporocarp, it is certain that no law of organization and succession of the parts can be formulated which will hold good of all the pileate fungi. There are probably some, as suggested above, in which the pileus and stipe primordia are organized before the primordium of the hymenium, and many others probably in which the stem is partially or quite well organized before there is even a primordium of the pileus, in which case the development would be in conformity with SCHMITZ's law given above. This is very likely the case with certain long, slender-stemmed species of Marasmius, of such plants as *Polyporus lucidus* Leys. (*Ganoderma lucida*), and others. But we must wait until the different types have been carefully studied from the very young stages in microtome sections.

FAYOD (*l. c.*), who studied a large number of Agaricaceae, formulates the general law that the pileus is organized first within the young primordium as a pileogenous layer (*couche piléogène*), which arises by internal differentiation, marked by the more rapid growth of the hyphal elements and their richer content in protoplasm. This layer is in the form of a shallow inverted bowl, convex above, concave below. This is surrounded on the sides and above by a thin layer

[25] Reproduction in *Coprinus radiatus*. Grevillea **4**:53–65. *pls.* 54–61. 1875.

which he calls the primordial cuticle (*cuticle primordiale*). From his description the primordial cuticle varies in character and probably in structure also, and it is difficult to accept his conception of a primordial cuticle as a homologous structure in the large series of forms to which he applies the term. For example, he recognizes three main types in the development of the Agaricaceae: 1st, the gymnocarpous forms; 2d, the angiocarpous forms; 3d, the endocarpous forms, and the primordial cuticle is present in all except in a very few of the gymnocarpous forms. In the gymnocarpous forms the primordial cuticle consists generally of a more dense layer of tissue underneath which the pileogenous layer is formed. The margin of this becomes the border of the pileus, and as it extends laterally it dislocates at this point the primordial cuticle, so that the primordium of the hymenium which now arises is of exogenous origin, thus giving rise to the gymnocarpous type. Examples are *Panus stipticus*, *Cantharellus cibarius*, *Marasmius rotula*, *Collybia racemosa*, etc. Thus while DEBARY believed the pileus in the gymnocarpous forms originated exogenously, FAYOD holds that it originates endogenously; but in discussing farther certain other forms he admits that the pileus is formed in the manner indicated by DEBARY, and he states that the discussion which he has raised here is more in regard to a principle than fact, and he would not have raised the question at all had it not been for the fact that his study of the angiocarpous forms had shown him the importance of the pileogenous layer.

In many of the angiocarpous forms the primordial cuticle would seem from his own description to be a different structure from what it is in the gymnocarpous forms, for he says: "The hyphae which emanate from the pileogenous layer do not reach the surface of the primordium. As a consequence the primordial cuticle, which acquires here a very considerable thickness, preserves its integrity and continues to increase up to the time of the formation of the lamellae and stipe, that is, up to the second period of development." Here he recognizes the primordial cuticle as identical with the universal veil, and he would call it general veil (*voile général*), although it is often formed in some cases by parts also of the subjacent tissue of the primordium, were it not for the fact that he wishes to place it in the same category as the non-integral element of the primordium

in the gymnocarpous forms which he considers a primordial cuticle.

The angiocarpous forms he further divides into two types, subangiocarpous and angiocarpous. In the subangiocarpous type the universal veil (*cuticule primordiale*), being continuous over the pileus and stem, forms the veil which is known as the "partial veil" or "marginal veil" of authors. He says (p. 286) it is probably characteristic of Flammula, Inocybe, Dermocybe, Hygrocybe, Psalliota, Lepiota, Psathyrella, Coprinus, and most of the Tricholomae. In the true angiocarpous types there is a cuticle of the pileus which is organized underneath the primordial cuticle or universal veil, so that at maturity the universal veil separates and forms floccose patches, or a volva, or may disappear by gelatinization. As examples he cites Agrocybe (*Pholiota praecox*, *Naucoria semiorbicularis*, etc.), Pholiotina (*Pholiota blattaria*, *P. togularis*, etc.), *Rozites* Karsten (*Pholiota caperata*), *Nemataloma* Karsten, some species of Panaeolus, Telamonia, and probably Locellina and Chitonia; the volva in the last three genera he considers to be only a very thick universal veil.

In the endocarpous forms the primordium of the fruit body is differentiated on the interior of a primitive bulb which he calls the primordial bulb (*bulbe primordial*), to which belong the greater number of species of Amanita, Volvaria, and some species of Phlegmacium. Since this type does not concern us here it will not be in place to discuss it.

From the foregoing it is seen that FAYOD places Psalliota (which includes *Agaricus campestris*) in his type of subangiocarpous forms. Among these forms he studied *Agaricus rubellus* Gillet (*Psalliota rubella*). While he does not describe the development of this species (his discussions of development are in the form of general conclusions), he says that it belongs to the subangiocarpous type, and his *fig. 4, pl. 7*, shows the primordial cuticle to consist of rather loose radiating threads connected on the sides where it extends down over the lamellae and stipe with the thicker portion covering the stem. Although FAYOD placed *Agaricus campestris* also in his subangiocarpous type, a study of these cultivated forms shows that it would belong to his true angiocarpous type because of the free universal veil entirely independent of the marginal veil, the universal veil eventually sepa-

rating into floccose patches on the surface of the pileus as in *Rozites caperata* (Pers.) Karsten (*Pholiota caperata* Pers.).

During the later period of growth and the beginning of elongation of the plant, the marginal veil increases in thickness and extent. It is entirely free from the lamellae, the hymenial cavity being quite distinct from the first and becoming greater by the expansion of the pileus and marginal veil, and also by the elongation of the portion of the stem above its attachment. The increase in the surface extent of the marginal veil is considerable and results in throwing the upper surface into radiate folds which are quite noticeable, especially in the well developed individuals. In the young primordium at the time of the organization of the parts of the carpophore the marginal veil is attached over a large part of the outer surface of the stem primordium, the lower end, perhaps that portion which corresponds to the bulb in other species, being free. It thus remains attached over the stem surface for a considerable period during growth. As the period of elongation advances, the veil begins to separate from the stem at the lower end and is gradually torn off and upward as the pileus expands and the stem elongates. The tension of the connecting fibers can very easily be seen between the stem and the under surface of the veil, and is well shown in *fig. 20*. It therefore forms a sheath over the stem except a short section of the lower end, and the portion above the marginal veil which is elongating. This sheath is loosened from below upward except at the upper point at attachment to the stem. The outer margin of the veil is attached to the rounded and thick margin of the pileus, and being of considerable thickness in these cultivated forms the lower edge of the veil is separated first from the outer surface of the pileus margin (*fig. 40*), and the inner upper edge is separated last from the inner surface of the pileus margin. The margin of the veil is therefore furrowed (*figs. 18, 19*). A thick marginal veil of this type is called a "double veil," a type which is very characteristic of certain other species of Agaricus where it is more highly developed, especially in *Agaricus arvensis*, where the lower portion of the veil splits radially. It is very striking in *Agaricus rodmani* Pk., where the forking of the veil extends almost to the stem.[26] In *Agaricus placomyces* Pk. the veil is often similar to

[26] See *fig. 17*, ATKINSON, Studies of American fungi, mushrooms, edible, poisonous, etc. 1900, 1901, Ithaca; 1903, New York.

that of *Agaricus arvensis* (*figs. 21, 22* in Studies Am. fungi, etc.), while in *Agaricus silvicola* and others the lower half of the marginal veil is often separated into patches (*l. c. fig. 20*). In the pasture or field forms of *Agaricus campestris* the marginal veil is thinner, but even here its double character is often manifest (*fig. 7, l. c.*).

The growth of the pileus which at first is strongly hyponastic becomes less so as the pileus expands. The upper surface gradually ceases to grow and the extension of the underlying part often tears the pileus cuticle into fibrous scales. The growth of the pileus gradually becomes epinastic, as the lower area and the hymenophore with the gills now grow more rapidly than the middle and upper portions. This causes the pileus to become plane, or in old specimens the margin itself becomes upturned. This peculiarity in the growth of the Agaricaceae during the period of elongation was supposed by some of the earlier botanists[27] to be due to the influence of light, for it was thought by them to be necessary that the hymenium should be turned up to the light. We now know that light is not necessary for the growth and ripening of many species. This partial eversion of the pileus in many species unquestionably serves a useful purpose in providing for the wider distribution of the spores, for they are more easily caught by currents of air as they leave the hymenium.

The order in which elongation of the different parts takes place is thus different from the order of their initiation in the young primordium. As has been shown in these cultivated forms of *Agaricus campestris*, the organization of the primordia of pileus, stem, hymenium, and marginal veil is practically simultaneous by the appearance of the hymenial primordium as an internal annular area; while the organization of the parts gradually proceeds and is also simultaneous to a certain degree. But the period of elongation of the parts after they have become organized, while overlapping to a certain extent, follows in succession. The marginal veil completes its period of elongation first, then the stem, followed by the pileus, and finally the hymenium.

One striking feature of the hymenium of these cultivated forms is that, so far as I have examined (the varieties Columbia, Alaska,

[27] See NEES VON ESENBECK, Das System der Pilze und Schwämme 179–187. 1816.

Bohemia, and others), the basidia are two-spored. I have several times observed this fact in the cultivated mushroom. The illustration of the hymenium of *Agaricus campestris* which I have used on two former occasions[28] was made from a cultivated variety. GOEBEL'S *fig. 90*[29] shows only two spores on the basidium of *Agaricus campestris*, and this was probably also made from a cultivated variety. I have on the other hand several times observed that in case of the normal field or pasture form of *Agaricus campestris* there are four spores on a basidium. The nuclear phenomena in the formation of the spores have not yet been thoroughly worked out in the two-spored forms of *Agaricus campestris*, but studies of C. E. LEWIS carried on in my laboratory seem to show that the normal number of four nuclei are first formed in the basidium, and that two of them degenerate. This has been very clearly shown by him to be the case in a new species of Amanita, *A. bisporigera* Atkinson.[30] Nor has it been shown how the two-spored forms of *Agaricus campestris* arise from the normal four-spored feral plant, or whether normal two-spored forms exist as constant types in the field under natural conditions of environment. I have found a two-spored Agaricus resembling in some respects certain of the cultivated forms of *Agaricus campestris* growing spontaneously in the open. On one occasion it was found in June about young trees in a lawn which had been mulched with horse manure. On another occasion the same species was found on the hillside of a wooded ravine (Cascadilla gorge) on the campus of Cornell University.

If there are two-spored forms of *Agaricus campestris* existing under natural conditions of environment which are constant and which present also other characters even slightly different, it would indicate that *Agaricus campestris* either is or recently has been passing through a mutating period, and that these forms are elementary species. Were the two-spored basidia the only differentiating character, such a form might in the sense of DEVRIES[31] be regarded as

[28] Studies and illustrations of mushrooms. I. Cornell Univ. Agr. Exp. Sta. Bull. 168. *fig. 189.* 1897; and Studies of Am. fungi, etc. (*l.*)

[29] Outlines of classification and special morphology, Eng. ed., 1887.

[30] LEWIS, C. E., The development of the spores in *Amanita bisporigera* Atkinson. BOT. GAZ. **41**:348–352. 1906.

[31] Species and varieties, their origin by mutation. 1905.

a variety, for it would seem that the four-spored quality or character is latent, since four nuclei are probably formed in the basidium in the normal manner but only two of them function. With regard to the cultivated forms of *Agaricus campestris* they probably represent also mutations either from *Agaricus campestris* or from some other species which has been confounded with it. Whether they are to be considered elementary species or varieties or retrograde varieties would depend upon their constancy or inconstancy, their stability or instability. They may be horticultural or domesticated varieties. Nevertheless it would seem that they have arisen by mutation. It is interesting to note in this connection that, whether species or varieties, if they have arisen by mutation their chances of becoming constant may be greater than in the case of plants which are well known to reproduce sexually. It is generally believed that the Agaricaceae are not reproduced by the cooperation of sexual organs. If this is true, and if there is no process similar to fertilization, mutations of these plants would escape one of the operations in nature against the constancy of new mutants in species capable of cross fertilization. Some students regard the fusion of the two primary nuclei in the basidium as an act of fertilization, but from what we know of the origin of these two nuclei the possibility of cross fertilization of individuals at this epoch of development is excluded, though it cannot at present be regarded as impossible at an earlier stage in their ontogeny. Of course the earlier ideas of fertilization in the Agaricaceae held in the time of BULLIARD,[32] who called the cystidia spermatic vessels and thought they squirted their juices on the seeds (spores) thus bringing about fertilization, or by CORDA[33] who regarded the cystidia as pollinaria and thought fertilization was brought about by the exudation of their fluid content to which the spores became attached and fertilized, are now unthinkable, as well as the notion of W. G. SMITH[34] as late as 1875, who believed that filaments growing out from the cystidia came in contact with the spores and fertilized

[32] BULLIARD, Histoire des champignons de la France **1**:39–66. 1791.

[33] CORDA, Berich. Ises. **6**:40. 1834; also Icones **3**:44. 1839. See also H. HOFFMANN, Pollinarien und Spermatien bei Agaricus. Bot. Zeit. **14**:137–148, 153–163. *pl. 15*. 1856.

[34] SMITH, W. G., Reproduction in *Coprinus radiatus*. Grevillea **4**:53–65. *pls. 54–61*. 1875.

them, and that hybrids between species were very commonly found where cystidia and spores from adjacent species fell to the ground, commingled, and brought about cross fertilization. But the last word may not yet have been said with reference to the possibility of a fertilization prior to or during the early stages of the organization of the primordium of the carpophore, like that proposed by OERSTED (*l. c.*) for *Agaricus* (*Crepidotus*) *variabilis*, or in some closely related manner.

However, the propagation of forms by spawn which is not obtained from the spores, as is practiced by DUGGAR,[35] would seem to be equivalent to vegetative propagation or budding, and thus might be of advantage in maintaining constancy in varieties, since they would not be subject to cross fertilization, though it is still a question if fertilization and cross fertilization take place in the Agaricaceae. If it does not, or if some process equivalent to it, especially cross fertilization, does not take place, the Agaricaceae, and in fact the Hymeniales, would be especially free from the production of hybrids, and the constancy of species or varieties arising by mutation would be correspondingly favored. In a number of species there are indications that mutation is now going on, or that these species have recently passed through a period of mutation, and some of these apparent mutants appear to be quite constant. On the other hand, there are many species which show great fluctuating variability due to varying conditions of food supply, moisture, substratum, etc.

CORNELL UNIVERSITY,
Ithaca, N. Y.

DESCRIPTION OF PLATES VII–XII.

Photomicrographs with Zeiss microscope except *fig. 11;* plate holder 360^{mm} from object on slide; photomicrographs and photographs by the author.

PLATE VII.

FIG. 1. Young carpophore, var. Alaska, undifferentiated; oc. 2, obj. 16^{mm}.

FIG. 2. Same as *fig. 1*, but with oc. 4, obj. 16^{mm}.

FIG. 3. Young carpophore, var. Columbia, with primordium of hymenium in earliest stages of endogenous origin; oc. 2, obj. 16^{mm}.

35 DUGGAR, B. M., The principles of mushroom growing and mushroom spawn-making. U. S. Dept. of Agr., Bureau of Plant Industry, Bull. 85. pp. 9–60. *pls. 1–7.* 1905.

FIG. 4. Young carpophore, var. Alaska, with endogenous primordium of hymenium a little more advanced than in *fig. 3;* universal veil distinct as a loose definite layer of tissue surrounding the carpophore; oc. 2, obj. 16^{mm}.

FIG. 5. Young carpophore, var. Columbia, showing endogenous primordium of hymenium a little more advanced than in *fig. 4;* universal veil as a very thin layer; oc. 2; obj. 16^{mm}.

PLATE VIII

FIG. 6. From young carpophore, var. Columbia; at stage when gill slit is just forming, showing sharp-pointed threads of primordium of hymenium pro jecting downward; also shows at the right the thin layer of universal veil; about same magnification as *fig. 7*, but not with Zeiss microscope.

FIG. 7. Same object as *fig. 6*, but showing only the gill slit and primordium of hymenium.

FIG. 8. Same as *figs. 6, 7*, but more highly magnified; oc. 6, obj. 3^{mm}.

PLATE IX.

FIG. 9. Young carpophore, var. Alaska, showing endogenous primordium of hymenium on one side, about the same stage as *fig. 4*, but higher magnification; oc. 4, obj. 16^{mm}.

FIG. 10. Young carpophore, var. Alaska, with endogenous primordium more advanced, showing definite and clear opening, the descending threads of the primordium of the lamellae, loose tissue of the inner veil, and primordium of stem cortex, outlined as an oblique area of younger threads rich in protoplasm, extending from opening obliquely downward and outward; oc. 6, obj. 3^{mm}.

FIG. 11. Portion of young carpophore, var. Columbia, showing gill slit; young lamellae in longitudinal section, inner veil of more open loose tissue; shows also how primordium of hymenium continues in its development as margin of pileus continues to grow; cortex of stem well-formed, showing as a more compact tissue over the surface to which the veil is attached; oc. 4, obj. 16^{mm}.

FIG. 12. Portion of young carpophore same age as *fig. 11* and from same plant, but cut somewhat obliquely so that a number of gills are shown in oblique section, otherwise as in *fig. 11*, but with less magnification; oc. 2, obj. 16^{mm}.

PLATE X.

FIG 13. Cluster of young carpophore, var. Columbia; numerous very young ones, several in the large button stage, showing the small white patches of the universal veil on the brown cuticle of the pileus.

FIG. 14. Another cluster of young carpophores, var. Columbia, showing numerous very small ones, and several of the small button stage, the universal veil separated into a few large white thin patches; rhizomorphs in the substratum.

PLATE XI.

FIG. 15. Longitudinal section of young carpophores magnified twice the real length, showing endogenous primordium of hymenium, gill slits, veil, and the primordium of cortex of stems.

FIG. 16. Real size; young carpophores, var. Columbia, showing rhizomorphs, the expanding young pileus and universal veil separated into patches; it is very distinct in the two plants where the universal veil is stretched between the two caps; at the right a few in longitudinal section.

FIG. 17. Young carpophore, var. Columbia, about half grown, real size, showing a few white patches of universal veil on the pileus, about midway of the short stem showing a ring which is the lower part of the double ring; in this case separated from the upper part and remaining on the stem as a distinct ring as in *Agaricus rodmani;* upper portion of the veil still attached from stem to margin of pileus which is as yet close against the stem.

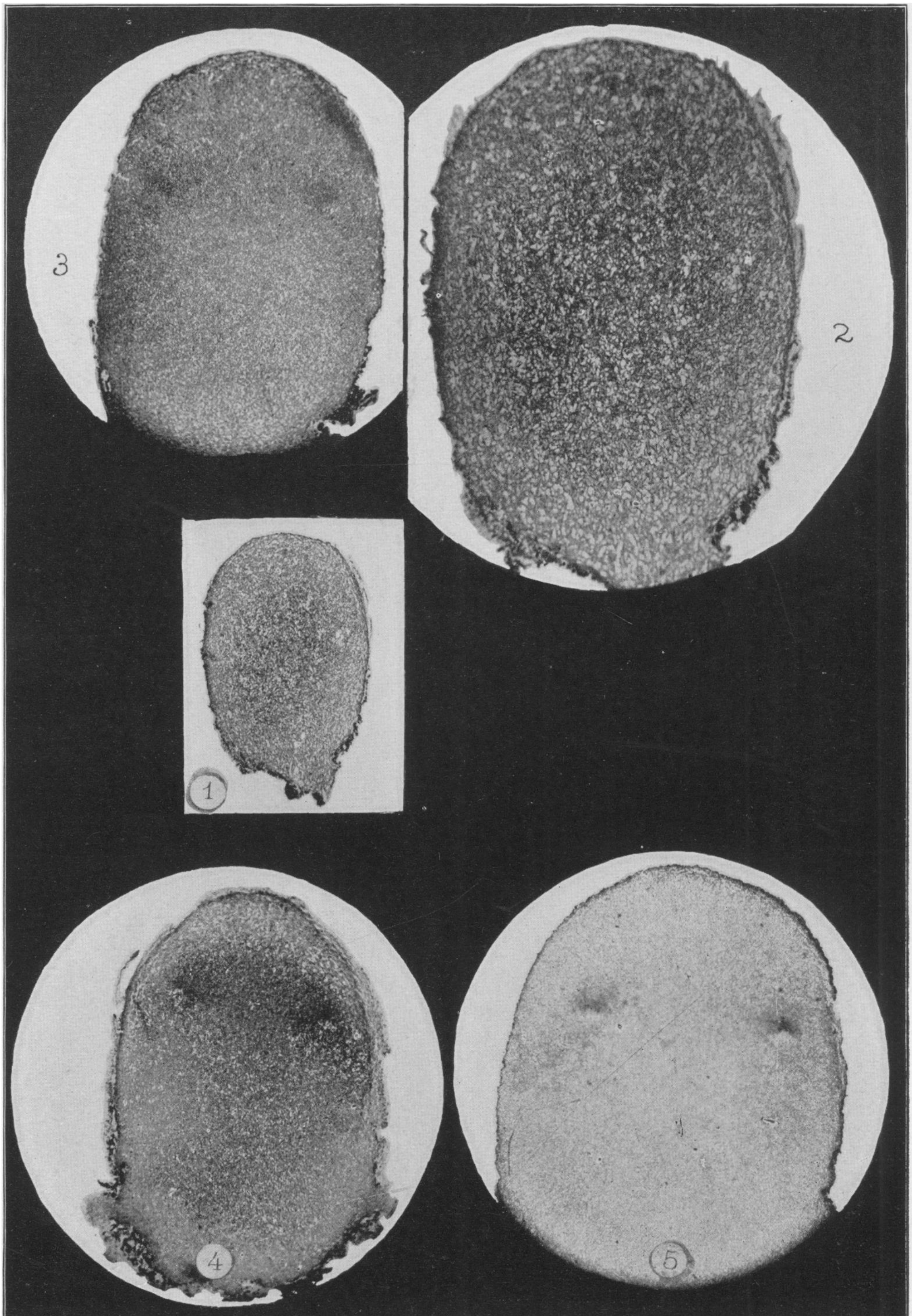
PLATE XII.

FIG. 18. Cluster of mature carpophores of *Agaricus campestris*, cult. var., showing patches of universal veil on pileus.

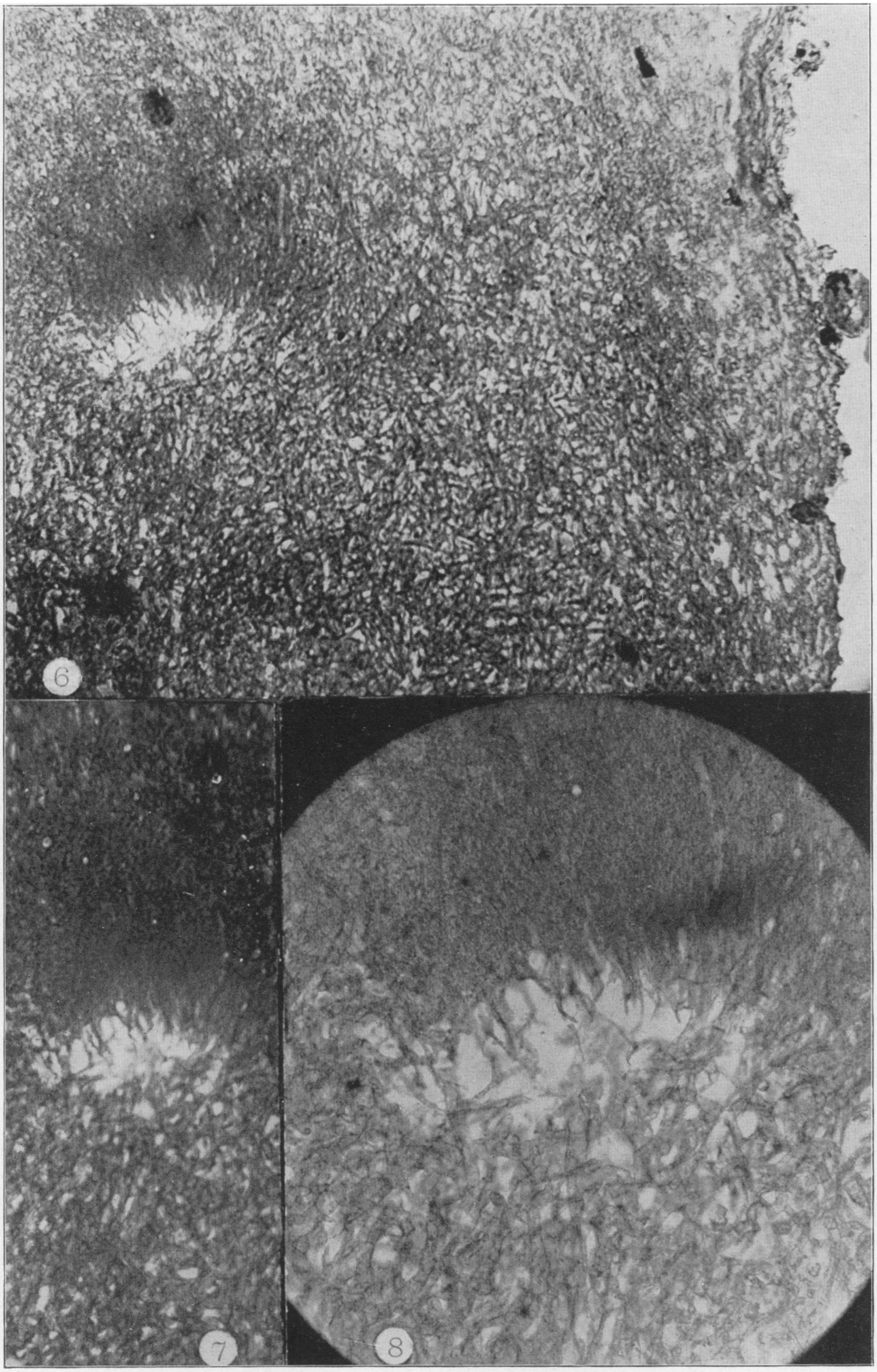
FIG. 19. Mature and nearly mature plants, var. Columbia, showing thick veil which forms a sterile margin on the edge of the pileus and a thick ring wrinkled or corrugated on the upper surface and the edge distinctly double.

FIG. 20. Slightly younger stage, also lower part of double veil as broken away from the outer surface of margin of pileus; upper part of double veil still attached to margin of pileus.

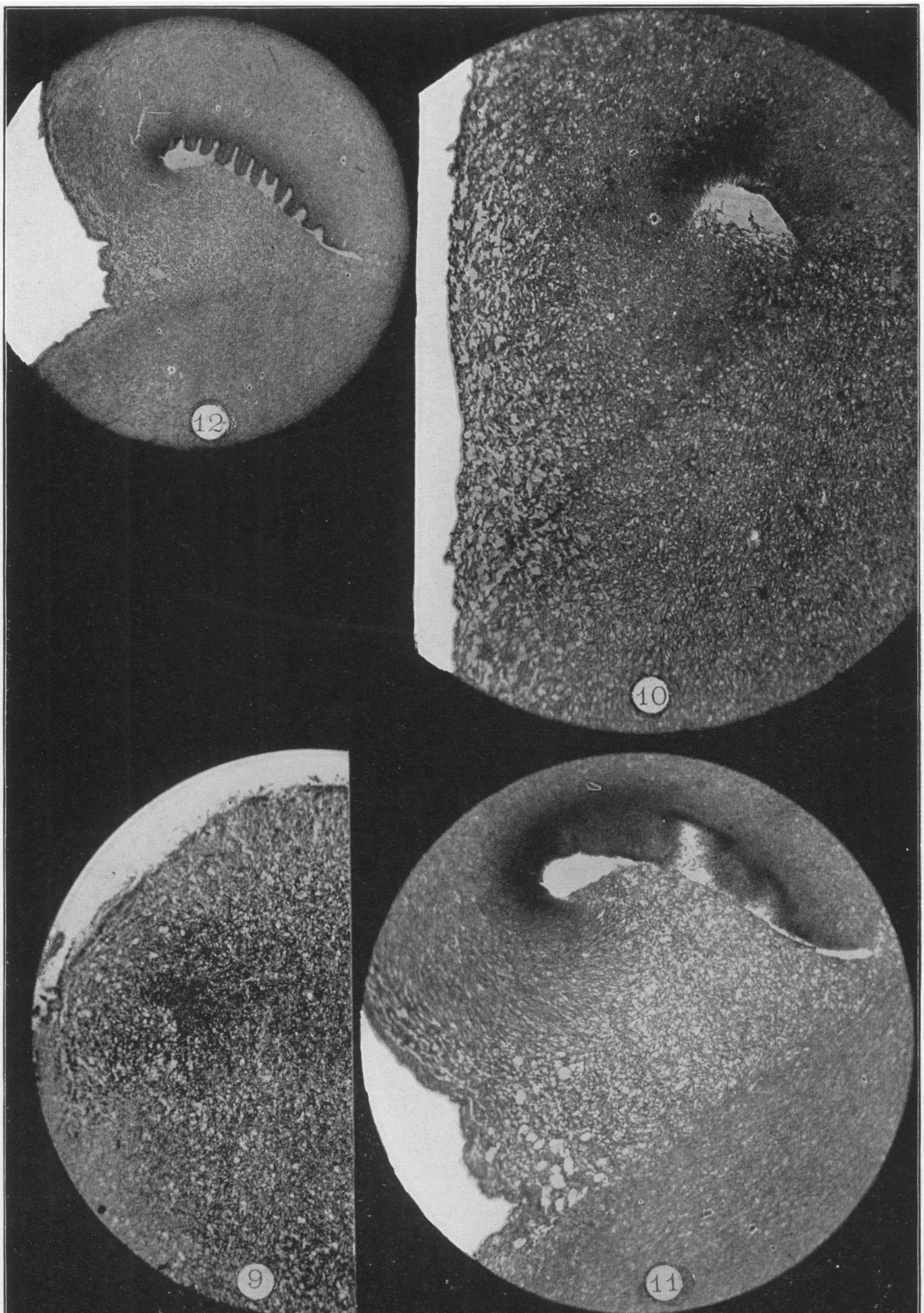
The material was selected and fixed in chromo-acetic acid by myself, and I am under many obligations to Dr. CHARLES E. LEWIS, who then took the material, carried it into paraffin, sectioned, and stained it.



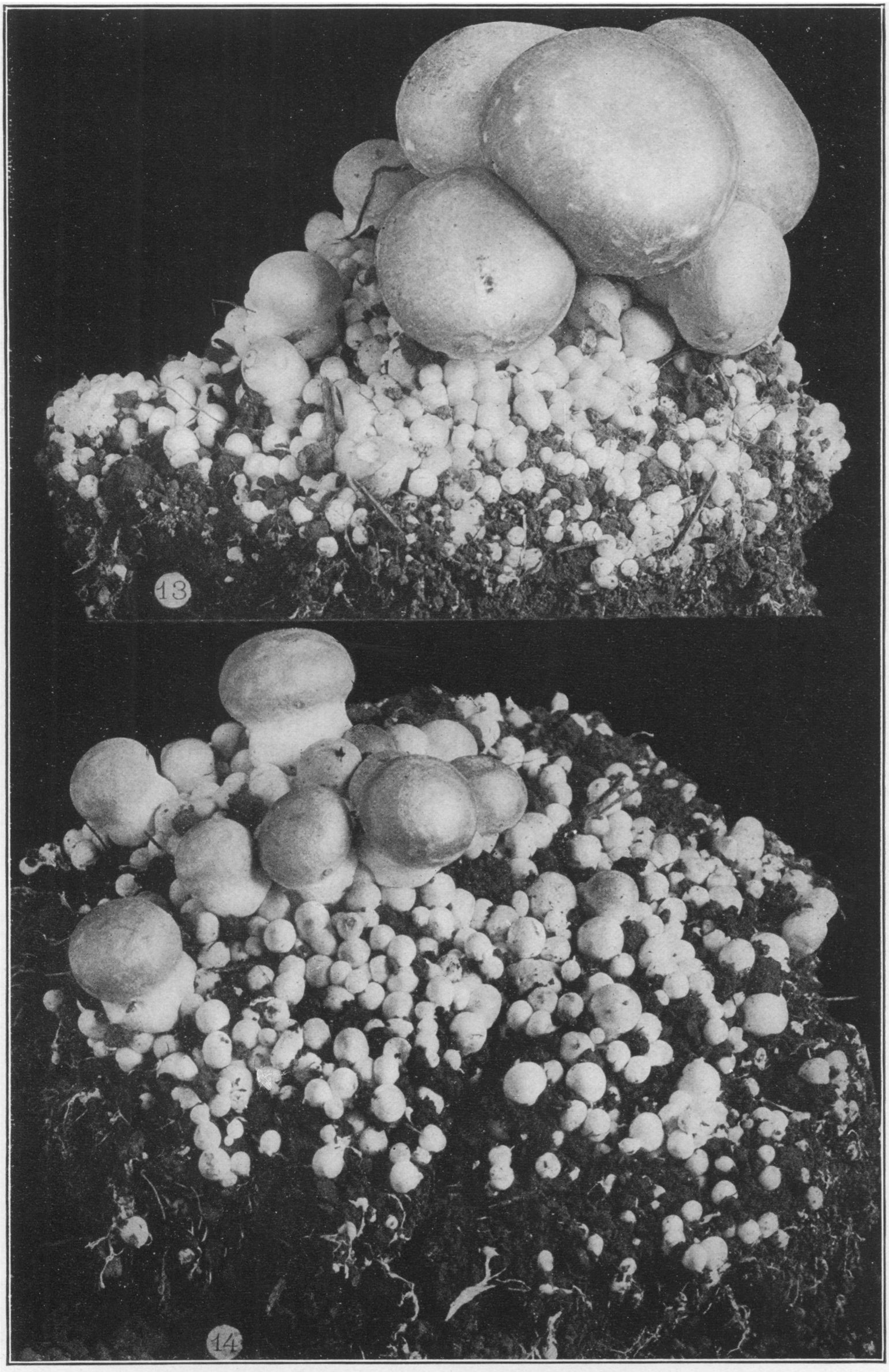
ATKINSON on AGARICUS



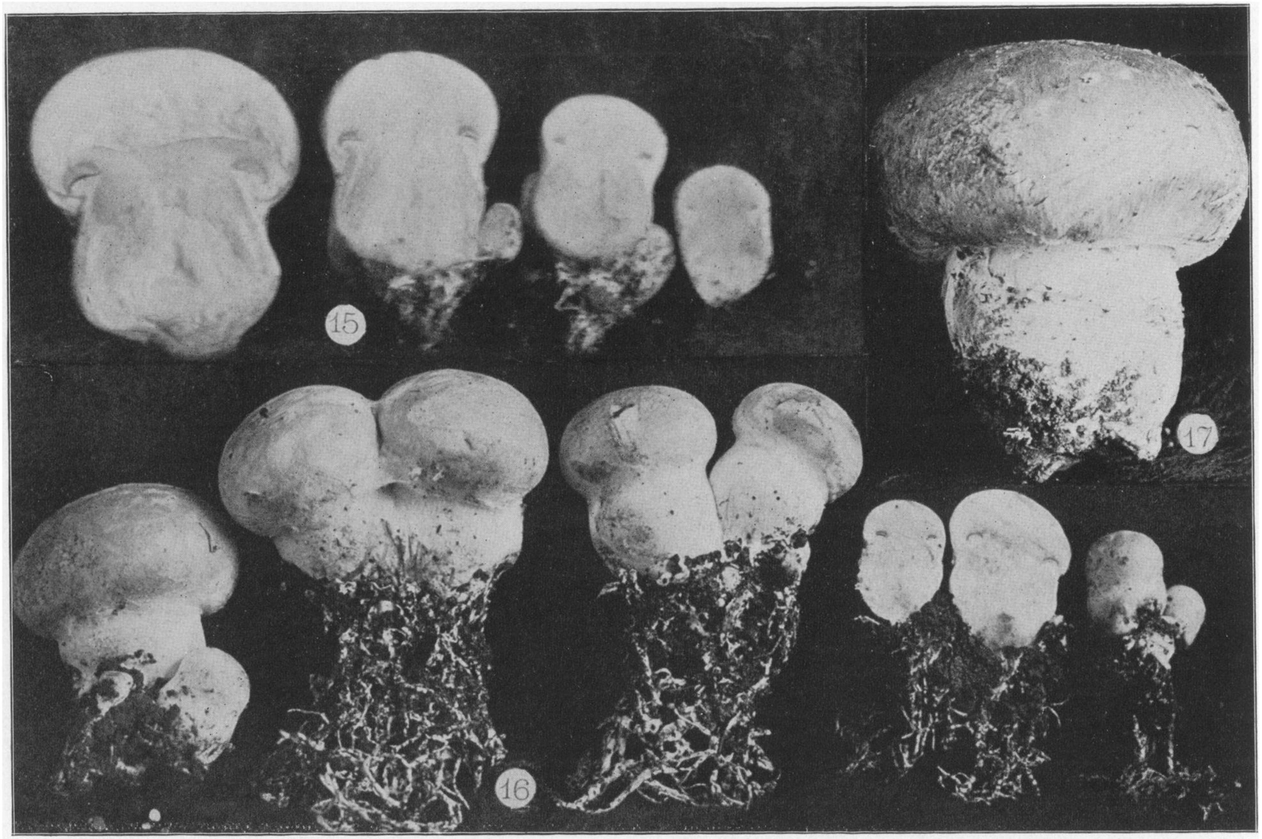
ATKINSON on AGARICUS



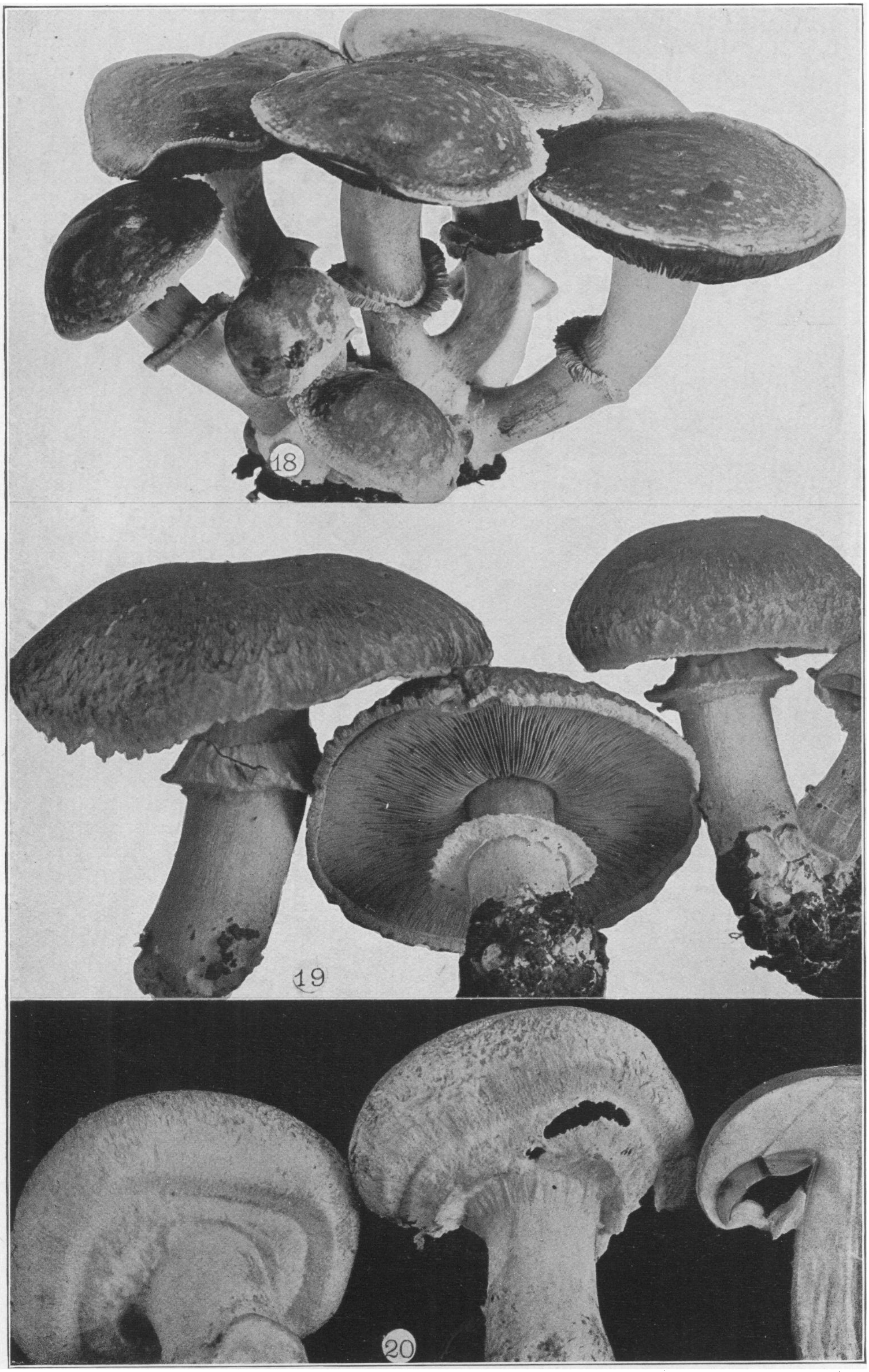
ATKINSON on AGARICUS



ATKINSON on AGARICUS



ATKINSON on AGARICUS



ATKINSON on AGARICUS